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DIALOG(R)File 351:Derwent WPI


007388666
WPI Acc No: 1988-022601/*198804*
XRAM Acc No: C88-009931
**Pharmaceutical with stabilised granulocytes colony stimulating factor - contains surfactant, saccharide, protein or high mol. wt. cpd. as stabiliser**
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Number of Countries: 023 Number of Patents: 036
Patent Family:

| Patent No | Kind | Date | Applicat No | Kind | Date | Week | |
|---|---|---|---|---|---|---|---|
| **DE 3723781** | **A** | **19880121** | **DE 3723781** | **A** | **19870717** | **198804** | **B** |
| **GB 2193631** | **A** | **19880217** | **GB 8716904** | **A** | **19870717** | **198807** | |
| FR 2601591 | A | 19880122 | FR 8710156 | A | 19870717 | 198811 | |
| NL 8701640 | A | 19880216 | NL 871640 | A | 19870713 | 198811 | |
| AU 8775665 | A | 19880121 | | | | 198812 | |
| NO 8702966 | A | 19880215 | | | | 198812 | |
| SE 8702907 | A | 19880119 | | | | 198812 | |
| DK 8703683 | A | 19880119 | | | | 198815 | |
| ZA 8705268 | A | 19880127 | ZA 875268 | A | 19870717 | 198818 | |
| HU 44941 | T | 19880530 | | | | 198825 | |
| JP 63146826 | A | 19880618 | JP 87178031 | A | 19870716 | 198830 | |
| JP 63146827 | A | 19880618 | JP 87178032 | A | 19870716 | 198830 | |
| JP 63146828 | A | 19880618 | JP 87178034 | A | 19870716 | 198830 | |
| JP 63152326 | A | 19880624 | JP 87178033 | A | 19870716 | 198831 | |
| PT 85343 | A | 19880729 | | | | 198835 | |
| BE 1000253 | A | 19880927 | BE 88787 | A | 19880927 | 198840 | |
| CN 8704963 | A | 19880224 | | | | 198915 | |
| CH 671157 | A | 19890815 | | | | 198937 | |
| ES 2010226 | A | 19891101 | ES 872106 | A | 19870717 | 199004 | |
| GB 2193631 | B | 19901121 | | | | 199047 | |
| IT 1218927 | B | 19900424 | | | | 199211 | |
| CA 1297007 | C | 19920310 | | | | 199216 | |
| NO 171828 | B | 19930201 | NO 872966 | A | 19870716 | 199310 | |
| IL 83220 | A | 19930131 | IL 83220 | A | 19870717 | 199311 | |
| KR 9304597 | B1 | 19930601 | KR 877804 | A | 19870718 | 199423 | |
| RU 2025120 | C1 | 19941230 | SU 4203033 | A | 19870717 | 199531 | |
| SE 503312 | C2 | 19960513 | SE 872907 | A | 19870717 | 199625 | |
| AT 8701775 | A | 19960815 | AT 871775 | A | 19870714 | 199637 | |
| DK 171308 | B | 19960902 | DK 873683 | A | 19870715 | 199641 | |
| JP 2577742 | B2 | 19970205 | JP 87178031 | A | 19870716 | 199710 | |
| JP 2577743 | B2 | 19970205 | JP 87178032 | A | 19870716 | 199710 | |
| AT 402259 | B | 19970215 | AT 871775 | A | 19870714 | 199713 | |
| JP 2629000 | B2 | 19970709 | JP 87178033 | A | 19870716 | 199732 | |
| NL 192917 | B | 19980105 | NL 871640 | A | 19870713 | 199807 | |
| PH 28830 | A | 19950314 | PH 35555 | A | 19870717 | 199844 | |
| DE 3723781 | C2 | 19990902 | DE 3723781 | A | 19870717 | 199939 | |

Priority Applications (No Type Date): JP 86169489 A 19860718; JP 86169486 A 19860718; JP 86169487 A 19860718; JP 86169488 A 19860718; JP 87178031 A 19870716; JP 87178032 A 19870716; JP 87178033 A 19870716
Patent Details:

| Patent No | Kind | Lan | Pg | Main IPC | Filing Notes |
|---|---|---|---|---|---|
| DE 3723781 | A | | 9 | | |
| NO 171828 | B | | | A61K-037/02 | Previous Publ. patent NO 8702966 |
| RU 2025120 | C1 | | 10 | A61K-009/14 | |
| DK 171308 | B | | | A61K-038/18 | Previous Publ. patent DK 8703683 |
| JP 2577742 | B2 | | 6 | A61K-038/00 | Previous Publ. patent JP 63146826 |
| JP 2577743 | B2 | | 6 | A61K-038/00 | Previous Publ. patent JP 63146827 |
| AT 402259 | B | | | A61K-038/19 | Previous Publ. patent AT 8701775 |
| JP 2629000 | B2 | | 5 | A61K-038/00 | Previous Publ. patent JP 63152326 |
| NL 192917 | B | | 11 | A61K-047/00 | |
| IL 83220 | A | | | A61K-037/02 | |
| KR 9304597 | B1 | | | A61K-037/02 | |
| SE 503312 | C2 | | | A61K-038/19 | |
| AT 8701775 | A | | | A61K-038/19 | |
| PH 28830 | A | | | A61K-031/715 | |
| DE 3723781 | C2 | | | A61K-038/16 | |

Abstract (Basic): DE 3723781 A

A pharmaceutical contains stabilised G-CSF (granulocytes colony stimulating factor) as active ingredient and at least one surfactant, saccharide, protein or a high mol. wt. cpd. Pref. the amt. of surfactant or saccharide is 1-10000 pts. wt. Pref. pt. wt. G-CSF. Pref. the surfactant is non-ionic (esp. a sorbitan ester glycerine ester, poly-glycerine ester, polyoxyethylene sorbital ester, polyoxyethylene-glycerine ester or polyethylene glycol ester of an aliphatic acid, polyoxyethylene polyoxypropylene alkyl ether, a hardened polyoxyethylated castor oil, a polyoxyethylated bees wax deriv. a polyoxyethylene lanoline deriv. or an aliphatic polyoxyethylene acid aride), anionic (esp. an alkylsulphate or alkylsulpho succinyl ester salt) or natural (esp. lecithin, sphingophospholipid or an ester of an aliphatic acid with sucrose.

USE/ADVANTAGE - G-CSF can be used to treat various infectious diseases, but is unstable and highly sensitive to changes in the environment e.g. temp., humidity, oxygen or UV light. The invention stabilises the G-CSF and protects it completely against loss of activity.

Title Terms: PHARMACEUTICAL; STABILISED; GRANULOCYTE; COLONY; STIMULATING; FACTOR; CONTAIN; SURFACTANT; SACCHARIDE; PROTEIN; HIGH; MOLECULAR; WEIGHT ; COMPOUND; STABILISED

Derwent Class: A96; B04

International Patent Class (Main): A61K-009/14; A61K-031/715; A61K-037/02; A61K-038/00; A61K-038/16; A61K-038/18; A61K-038/19; A61K-047/00

International Patent Class (Additional): A61K-009/08; A61K-031/70; A61K-035/00; A61K-037/00; A61K-037/04; A61K-037/12; A61K-037/43; A61K-038/17; A61K-045/05; A61K-047/10; A61K-047/14; A61K-047/20; A61K-047/24; A61K-047/26; A61K-047/32; A61K-047/34; A61K-047/36; A61K-047/42; C12N-000/00

File Segment: CPI

(12) UK Patent Application (19) GB (11) 2 193 631 (13) A

(43) Application published 17 Feb 1988

(21) Application No 8716904

(22) Date of filing 17 Jul 1987

(30) Priority data
(31) 61/169486 (32) 18 Jul 1986 (33) JP
61/169487 18 Jul 1986
61/169488 18 Jul 1986
61/169489 18 Jul 1986

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(51) INT CL⁴
A61K 37/02 37/43

(52) Domestic classification (Edition J):
A5B 180 230 231 232 23X 23Y 316 319 31X 31Y 341 343 34Y 351 35Y JB
U1S 2410 A5B

(56) Documents cited
GB A 2016477

(58) Field of search
A5B
Selected US specifications from IPC sub-class A61K

# (54) Stable granulocyte colony stimulating factor composition

(57) A stable granulocyte colony stimulating factor-containing pharmaceutical preparation contains, in addition to the active agent, at least one substance selected from a pharmaceutically acceptable surfactant, saccharide, protein and high-molecular weight compound.

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SPECIFICATION

**Stable pharmaceutical preparation containing granulocyte colony stimulating factor and process for producing the same**

The present invention relates to a pharmaceutical preparation containing a granulocyte colony stimulating factor. In particular, the present invention relates to a stabilized pharmaceutical preparation containing a granulocyte colony stimulating factor that is protected against loss or inactivation of the active component (i.e., granulocyte colony stimulating factor) due to adsorption on the wall of a container in which the preparation is put, or to association, polymerization or oxidation of said component.

Chemotherapy has been undertaken as one method for treating a variety of infectious diseases but it has recently been found that chemotherapy causes some serious clinical problems such as the generation of drug-resistant organisms, change of causative organisms, and high side effects. In order to avoid these problems associated with chemotherapy involving the use of therapeutic agents such as antibiotics and bactericides, attempts are being made to use a substance that activates the prophylactic capabilities of the host of an infection-causing organism and thereby providing a complete solution to the aforementioned problems of chemotherapy. Of the various prophylactic capabilities of the host, the phagocytic bactericidal action of leucocytes is believed to cause the strongest influence in the initial period of bacterial infection and it is therefore assumed to be important to enhance the infection protecting capabilities of the host by promoting the growth of neutrophiles and their differentiation into the mature state. A granulocyte colony stimulating factor (G-CSF) is one of the very useful substances that exhibit such actions and the same assignee of the present invention previously filed a patent application on an infection protecting agent using G-CSF (Japanese Patent Application No. 23777/1985).

As mentioned above, chemotherapy as currently practiced involves various unavoidable problems and intensive efforts are being made to use a drug substance that is capable of activating the prophylactic functions of the host or the person who has been infected.

Needless to say, G-CSF displays by itself the ability to activate the prophylactic functions of the host and it has also been found that G-CSF exhibits greater therapeutic effects in clinical applications if it is used in combination with a substance that activates the prophylactic capabilities of the host.

G-CSF is used in a very small amount and a pharmaceutical preparation containing 0.1—500 μg (preferably 5—50 μg) of G-CSF is usually administered at a dose rate of 1—7 times a week per adult. However, G-CSF has a tendency to be adsorbed on the wall of its container such as an ampule for injection or a syringe. Therefore, if the drug is used as an injection in such a form as an aqueous solution, it will be adsorbed on the wall of its container such as an ampule or a syringe. This either results in the failure of G-CSF to fully exhibit its activity as a pharmaceutical agent or necessitates the incorporation of G-CSF in a more-than-necessary amount making allowance for its possible loss due to adsorption.

In addition, G-CSF is labile and highly susceptible to environmental factors such as temperature, humidity, oxygen and ultraviolet rays. By the agency of such factors, G-CSF undergoes physical or chemical changes such as association, polymerization and oxidation and suffers a great loss in activity. These phenomenon make it difficult to ensure complete accomplishment of a therapeutic act by administering a very small amount of G-CSF in a very exact manner.

It is therefore necessary to develop a stable pharmaceutical preparation of G-CSF that is fully protected against a drop in the activity of its effective component. This is the principal object of the present invention which provides a stable pharmaceutical preparation of G-CSF.

The present inventors conducted intensive studies in order to enhance the stability of a G-CSF containing pharmaceutical preparation and found that this object can effectively be attained by addition of a pharmaceutically acceptable surfactant, saccharide, protein or high-molecular weight compound.

Therefore, the stable G-CSF containing pharmaceutical preparation of the present invention is characterized by containing both G-CSF and at least one substance selected from the group of a pharmaceutically acceptable surfactant, saccharide, protein and high-molecular weight compound.

The G-CSF to be contained in the pharmaceutical preparation of the present invention can be obtained by any of the methods such as those described in the specifications of Japanese Patent Application Nos. 153273/1984, 269455/1985, 269456/1985, 270838/1985 and 270839/1985. For example, a human G-CSF can be prepared either by cultivating a cell strain (CNCM Accession Number I-315 or I-483) collected from tumor cells of patients with oral cavity cancer, or by expressing a recombinant DNA (which has been prepared by the agency of a human G-CSF encoding gene) in an appropriate host cell (e.g. *E. coli*, C 127 cell or ovary cells of a Chinese hamster).

Any human G-CSF that has been purified to high degree may be employed as the G-CSF to be contained in the pharmaceutical preparation of the present invention. Preferable human G-CSFs

are ones obtained by isolation from the supernatant of the culture of a human G-CSF producing cell, and a polypeptide or glycoprotein having the human G-CSF activity that is obtained by transforming a host with a recombinant vector having incorporated therein a gene coding for a polypeptide having the human G-CSF activity.

Two particularly preferable examples of human G-CSF are shown below:

(1) human G-CSF having the following physicochemical properties:

i) molecular weight: about 19,000 ± 1,000 as measured by electrophoresis through a sodium dodecylsulfate—polyacrylamide gel;

ii) isoelectric point: having at least one of the three isoelectric points, pI = 5.5 ± 0.1, pI = 5.8 ± 0.1, and pI = 6.1 ± 0.1;

iii) ultraviolet absorption: having a maximum absorption at 280 nm and a minimum absorption at 250 nm;

iv) amino acid sequence of the 21 residues from N terminus: $H_2N$-Thr-Pro-Leu-Gly-Pro-Ala-Ser-Ser-Leu-Pro-Gln-Ser-Phe-Leu-Leu-Lys-Cys-Leu-Glu-Gln-Val

(2) human G-CSF containing either a polypeptide having the human granulocyte stimulating factor activity which is represented by all or part of the amino acid sequence shown below, or a glycoprotein having both said polypeptide and a sugar chain portion:

$(Met)_n$Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro
Gln Ser Phe Leu Leu Lys Cys Leu Glu Gln Val
Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln
Glu Lys Leu (Val Ser Glu)$_m$Cys Ala Thr Tyr Lys
Leu Cys His Pro Glu Glu Leu Val Leu Leu Gly
His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser
Ser Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly
Cys Leu Ser Gln Leu His Ser Gly Leu Phe Leu
Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu
Gln Leu Asp Val Ala Asp Phe Ala Thr Thr Ile
Trp Gln Gln Met Glu Glu Leu Gly Met Ala Pro
Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala
Phe Ala Ser Ala Phe Gln Arg Arg Ala Gly Gly
Val Leu Val Ala Ser His Leu Gln Ser Phe Leu
Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala
Gln Pro

(provided that m is 0 or 1; and n is 0 or 1).

For details of the method for preparing these two types of G-CSF, see the specification of Japanese Patent Application Nos. 153273/1984, 269455/1985, 269456/1985, 270838/1985 and 270839/1985, all having been filed by the assignee of the present invention.

Another method that can be employed consists of performing fusion of a G-CSF producing cell with a self-proliferating malignant tumor cell and cultivating the resulting hybridoma in the presence or absence of mytogen.

The human G-CSF containing solution obtained may be stored in a frozen state after being further purified and concentrated, as required, by any known technique. Alternatively, the solu-

tion may be stored after being dehydrated by such means as freeze-drying.

All of the human G-CSFs thus prepared can be processed as specified by the present invention in order to attain stable G-CSF containing pharmaceutical preparations.

Typical examples of the surfactant that is used to attain the stable G-CSF containing pharmaceutical preparation of the present invention are listed below: nonionic surfactants with HLB of 6—18 such as sorbitan aliphatic acid esters (e.g. sorbitan monocaprylate, sorbitan monolaurate and sorbitan monopalmitate), glycerin aliphatic acid esters (e.g. glycerin monocaprylate, glycerin monomyristate, and glycerin monostearate), polyglycerin aliphatic acid esters (e.g. decaglyceryl monostearate, decaglyceryl distearate and decaglyceryl monolinoleate), polyoxyethylene sorbitan aliphatic acid esters (e.g. polyoxyethylene sorbitan monolaurate, polyoxyethylene sorbitan monooleate, polyoxyethylene sorbitan monostearate, polyoxyethylene sorbitan monopalmitate, polyoxyethylene sorbitan trioleate, and polyoxyethylene sorbitan tristearate), polyoxyethylene sorbitol aliphatic acid esters (e.g. polyoxyethylene sorbitol tetrastearate and polyoxyethylene sorbitol tetraoleate), polyethylene glycerin aliphatic acid esters (e.g. polyoxyethylene glyceryl monostearate), polyethylene glycol aliphatic acid esters (e.g. polyethylene glycol distearate), polyoxyethylene alkyl ethers (e.g. polyoxyethylene lauryl ether), polyoxyethylene polyoxypropylene alkyl ethers (e.g. polyoxyethylene polyoxypropylene glycol ether, polyoxyethylene polyoxypropylene propyl ether, and polyoxyethylene polyoxypropylene cetyl ether), polyoxyethylene alkylphenyl ethers (e.g. polyoxyethylene nonylphenyl ether), polyoxyethylated castor oil, polyoxyethylated hardened castor oil (polyoxyethylated hydrogenated castor oil), polyoxyethylated beeswax derivatives (e.g. polyoxyethylated sorbitol beeswax), polyoxyethylene lanolin derivatives (e.g. polyoxyethylene lanolin), and polyoxyethylene aliphatic acid amides (e.g. polyethylene stearic acid amide); nonionic surfactants such as alkyl sulfuric acid salts having a $C_{10}$—$C_{18}$ alkyl group (e.g. sodium cetyl sulfate, sodium lauryl sulfate and sodium oleyl sulfate), polyoxyethylene alkyl ether sulfuric acid salts wherein the average molar number of ethylene oxide addition is 2—4 and the alkyl group has 10—18 carbon atoms (e.g. polyoxyethylene sodium lauryl sulfate), salts of alkyl sulfosuccinate esters wherein the alkyl group has 8 -18 carbon atoms (e.g. sodium lauryl sulfosuccinate ester); and natural surfactants such as lecithin, glycerophospholipid, sphingophospholipid (e.g. sphingomyelin), and sucrose aliphatic acid esters wherein the aliphatic acid has 12—18 carbon atoms. These surfactants may of course be used either independently or in admixture.

The surfactants listed above are preferably used in amounts of 1—10,000 parts by weight per part by weight of G-CSF.

The saccharide to be used in making the stable G-CSF containing pharmaceutical preparation of the present invention may be selected from among monosaccharides, oligosaccharides, and polysaccharides, as well as phosphate esters and nucleotide derivatives thereof so long as they are pharmaceutically acceptable. Typical examples are listed below: trivalent and higher sugar alcohols such as glycerin, erythritol, arabitol, xylitol, sorbitol, and mannitol; acidic sugars such as glucuronic acid, iduronic acid, neuraminic acid, galacturonic acid, gluconic acid, mannuronic acid, ketoglycolic acid, ketogalactonic acid and ketogulonic acid; hyaluronic acid and salts thereof, chondroitin sulfate and salts thereof, heparin, inulin, chitin and derivatives thereof, chitosan and derivatives thereof, dextrin, dextran with an average molecular weights of 5,000 - 150,000, and alginic acid and salts thereof. All of these saccharides may be used with advantage either independently or in admixture.

The saccharides listed above are preferably used in amounts of 1—10,000 parts by weight per part by weight of G-CSF.

Typical examples of the protein to be used in making the stable G-CSF containing pharmaceutical preparation of the present invention include human serum albumin, human serum globulin, gelatin, acid-treated gelatin (average mol. wt. = 7,000—100,000), alkali-treated gelatin (average mol. wt. = 7,000—100,000), and collagen. Needless to say, these proteins may be used either independently or in admixture.

The proteins listed above are preferably used in amounts of 1—20,000 parts by weight per part by weight of G-CSF.

Typical examples of the high-molecular weigh compound to be used in making the stable G-CSF containing pharmaceutical preparation of the present invention include: natural polymers such as hydroxypropyl cellulose, hydroxymethyl cellulose, sodium carboxymethyl cellulose, and hydroxyethyl cellulose; and synthetic polymers such as polyethylene glycol (mol. wt. = 300—6,000), polyvinyl alcohol (mol. wt. = 20,000—100,000), and polyvinylpyrrolidone (mol. wt. = 20,000—100,000). Needless to say, these high-molecular weight compounds may be used either alone or in combination.

The high-molecular weight compounds listed above are desirably used in amounts of 1—20,000 parts by weight per part by weight of G-CSF.

In addition to the surfactant, saccharide, protein or high-molecular weight compound described above, at least one member selected from the group consisting of an amino acid, a sulfureous reducing agent and an antioxidant may also be incorporated in making the G-CSF containing pharmaceutical preparation of the present invention. Illustrative amino acids include glycine,

threonine, tryptophan, lysine, hydroxylysine, histidine, arginine, cysteine, cystine, and methionine. Illustrative sulfureous reducing agents include: N-acetylcysteine, N-acetylhomocysteine, thioctic acid, thiodiglycol, thioethanolamine, thioglycerol, thiosorbitol, thioglycolic acid and salts thereof, sodium thiosulfate, sodium hydrogensulfite, sodium pyrosulfite, sodium sulfite, thiolactic acid, dithiothreitol, glutathione, and a mild sulfureous reducing agent having a sulfhydryl group such as a $C_1$—$C_7$ thioalkanoic acid. Illustrative anti-oxidants include erythorbic acid, dibutylhydroxytoluene, butylhydroxyanisole, dl-α-tocopherol, tocopherol acetate, L-ascorbic acid and salts thereof, L-ascorbic acid palmitate, L-ascorbic acid stearate, triamyl gallate, propyl gallate and chelating agents such as disodium ethylenediaminetetraacetate (EDTA), sodium pyrophosphate and sodium metaphosphate.

The above-listed amino acids, sulfureous reducing agents and antioxidants or mixtures thereof are preferably used in amounts of 1—10,000 parts by weight per part by weight of G-CSF.

For the purpose of formulating the stable G-CSF containing preparation of the present invention in a suitable dosage form, one or more the following agents may be incorporated: a diluent, a solubilizing aid, an isotonic agent, an excipient, a pH modifier, a soothing agent, and a buffer.

The stabilized G-CSF pharmaceutical preparation of the present invention may be formulated either for oral administration or for parenteral administration such as by injection applied in various ways, and a variety of dosage forms may be employed depending upon the specific mode of administration. Typical dosage forms include: those intended for oral administration such as tablets, pills, capsules, granules and suspensions; solutions, suspensions and freeze-dried preparations principally intended for intravenous injection, intramuscular injection, subcutaneous injection and intracutaneous injection; and those intended for transmucosal administration such as rectal suppositories, nasal drugs, and vaginal suppositories.

According to the present invention, at least one substance selected from the group consisting of a surfactant, a saccharide, a protein or a high-molecular weight compound is added to a G-CSF containing pharmaceutical preparation so that it is prevented from being adsorbed on the wall of its container or a syringe while at the same time, it remains stable over a prolonged period of time.

The detailed mechanism by which the substances mentioned above stabilized G-CSF or prevent it from being adsorbed is yet to be clarified. In the presence of a surfactant, the surface of G-CSF which is a hydrophobic protein would be covered with the surfactant to become solubilized so that the G-CSF present in a trace amount is effectively prevented from being adsorbed on the wall of its container or a syringe. A saccharide or hydrophilic high-molecular weight compound would form a hydrated layer between G-CSF and the adsorptive surface of the wall of its container or a syringe, thereby preventing adsorption of G-CSF in an effective manner. A protein would compete with G-CSF for adsorption on the wall of its container or a syringe, thereby effectively inhibiting adsorption of G-CSF.

Besides the prevention of G-CSF adsorption, the substances mentioned above would also contribute to the prevention of association or polymerization of the molecules of G-CSF. In the presence of a surfactant, saccharide, protein or high-molecular weight compound, the individual molecules of G-CSF are dispersed in these substances and the interaction between the G-CSF molecules is sufficiently reduced to cause a significant decrease in the probability of their association or polymerization. In addition, these substances would retard the autoxidation of G-CSF that is accelerated under high temperature or humidity or prevent G-CSF from being associated or polymerized as a result of its autoxidation. These effects of retarding autoxidation of G-CSF or preventing it from being associated or polymerized would be further enhanced by addition of an amino acid, a sulfureous reducing agent or an antioxidant.

The problems described above are particularly noticeable in solutions for injection and in suspensions but they also occur during the process of formulating G-CSF in other dosage forms such as tablets. The addition of surfactants, saccharides, proteins or high-molecular weight compounds is also effective in this latter case.

Through the addition of at least one substance selected from the group consisting of a surfactant, saccharide, protein and a high-molecular weight compound, G-CSF is highly stabilized and maintains its activity for a prolonged period of time, as will be demonstrated in the examples that follow. To attain these results, the amount of each of these substances, in particular its lower limit, is critical and the following ranges are desirable: 1—10,000 parts by weight of surfactant, 1—10,000 parts by weight of saccharide, 1—20,000 parts by weight of protein, and 1—20,000 parts by weight of high-molecular weight compound, per 1 part by weight of G-CSF.

According to the present invention, a surfactant, a saccharide, a protein and/or a high-molecular weight compound is used in a specified concentration and this is effective not only in preventing G-CSF from being adsorbed on the wall of its container or a syringe but also in enhancing the stability of a G-CSF containing pharmaceutical preparation. As a result, it becomes possible to ensure the administration of a small but highly precise dose of G-CSF to patients; since G-CSF is costly, its efficient utilization will lead to lower costs for the production of G-CSF

containing pharmaceutical preparations.

The following examples are provided for the purpose of further illustrating the present invention but are in no sense to be taken as limiting. In these examples, the residual activity of G-CSF was determined by one of the following methods.

(a) *Soft agar method using mouse bone marrow cells*:

A horse serum (0.4 ml), 0.1 ml of the sample, 0.1 ml of a C3H/He (female) mouse bone marrow cell suspension ($0.5 - 1 \times 10^5$ nuclear cells), and 0.4 ml of a modified McCoy's 5A culture solution containing 0.75% of agar were mixed, poured into a plastic dish for tissue culture (35 $mm^\phi$), coagulated, and cultured for 5 days at 37°C in 5% $CO_2$/95% air and at 100% humidity. The number of colonies formed was counted (one colony consisting of at least 50 cells) and the activity was determined with one unit being the activity for forming one colony.

The modified McCoy's 5A culture solution used in the method (a) was prepared by the following procedures.

*Modified McCoy's 5A culture solution (double concentration)*

Twelve grams of McCoy's 5A culture solution (Gibco), 2.55 g of MEM amino acid-vitamin medium (Nissui Seiyaku Co., Ltd.), 2.18 g of sodium bicarbonate and 50,000 units of potassium penicillin G were dissolved twice in 500 ml of distilled water and the solution was aseptically filtered through a Millipore filter (0.22 μm).

(b) *Reverse-phase high-performance liquid chromatography*:

Using a reverse-phase C8 column (4.6 mm × 300 mm; 5 μm) and an n-propanol/trifluoroacetic acid mixture as a mobile phase, the residual activity of G-CSF (injected in an amount equivalent to 1 μg) was determined under the following gradient conditions:

| Time (sec) | Solvent (A) | Solvent (B) | Gradient |
|---|---|---|---|
| 0 | 100% | 0% | linear |
| 15 | 0% | 100% | linear |
| 25 | 100% | 0% | |

Solvent (A): 30% n-propanol and 0.1% trifluoroacetic acid
Solvent (B): 60% n-propanol and 0.1% trifluoroacetic acid

Detection was conducted at a wavelength of 210 nm and the percentage of the residual G-CSF activity was calculated by the following formula:

$$\text{Residual G-CSF activity (\%)} = \frac{\text{the residual amount of G-CSF after the lapse of a given time}}{\text{the initial amount of G-CSF}} \times 100$$

The residual amount of G-CSF as determined by this method correlated very well with the result attained in measurement by the soft agar method (a) using mouse bone marrow cells.

*Example 1*

To 5 μg of G-CSF, one of the stabilizing agents listed in Table 1 was added and the mixture was aseptically dissolved in a 20 mM buffer solution (containing 100 mM sodium chloride; pH 7.4) to make a pharmaceutical preparation containing 5 μg of G-CSF per ml, which was then freezer-dried. The time-dependent change in G-CSF activity was measured by method (a) and the results are shown in Table 1. The term "activity (%)" in the table represents the residual activity of G-CSF relative to the initial unit and is defined by the following formula:

$$\text{Activity (\%)} = \frac{\text{activity unit after the lapse of a given time}}{\text{initial activity unit}} \times 100$$

Freeze-drying was conducted by the following procedures:

The G-CSF solution containing a stabilizing agent was put into a sterile sulfa-treated glass vial, frozen at −40°C or below for 4 hours, subjected to primary drying by heating from −40°C to 0°C over a period of 48 hours with the pressure increased from 0.03 to 0.1 torr, then to secondary during by heating from 0°C to 20°C for a period of 12 hours with the pressure

increased from 0.03 to 0.08 torr; thereafter, the interior of the vial was filled with a sterile dry nitrogen gas to attain an atmospheric pressure and the vial was plugged with a freeze-drying rubber stopper, then sealed with an aluminum cap.

Table 1

| Stabilizing agent | Amount (parts by weight) | Activity (%) | |
|---|---|---|---|
| | | After storage at 4°C for 6 months | After storage at 37°C for 1 month |
| xylitol | 10,000 | 92 | 86 |
| mannitol | 10,000 | 91 | 85 |
| glucuronic acid | 10,000 | 86 | 82 |
| hyaluronic acid | 2,000 | 92 | 89 |
| dextran (m.w. 40,000) | 2,000 | 95 | 90 |
| heparin | 5,000 | 85 | 80 |
| chitosan | 2,000 | 93 | 91 |
| alginic acid | 2,000 | 90 | 90 |
| human serum albumin | 1,000 | 98 | 99 |
| human serum globulin | 1,000 | 98 | 95 |
| acid-treated gelatin | 2,000 | 97 | 95 |
| alkali-treated gelatin | 1,000 | 99 | 96 |
| collagen | 2,000 | 95 | 90 |
| polyethylene glycol (m.w. 4,000) | 10,000 | 94 | 90 |
| hydroxypropyl cellulose | 1,000 | 98 | 94 |
| sodium carboxymethyl cellulose | 1,000 | 88 | 80 |
| hydroxymethyl cellulose | 5,000 | 92 | 90 |
| polyvinyl alcohol (m.w. 50,000) | 2,000 | 96 | 95 |
| polyvinylpyrrolidone (m.w. 50,000) | 2,000 | 95 | 94 |
| human serum albumin<br>mannitol<br>cysteine | 2,000<br>2,000<br>100 | 100 | 97 |

Table 1 (cont'd)

| Stabilizing agent | Amount (parts by weight) | Activity (%) | |
|---|---|---|---|
| | | After storage at 4°C for 6 months | After storage at 37°C for 1 month |
| human serum albumin<br>polyoxyethylene sorbitan monolaurate<br>mannitol | 2,000<br>100<br>2,000 | 99 | 96 |
| human serum albumin<br>hydroxypropyl cellulose<br>dextran (m.w. 40,000) | 2,000<br>500<br>2,000 | 98 | 92 |
| polyoxyethylene sorbitan monolaurate<br>sorbitol | 100<br>2,000 | 98 | 96 |
| polyoxyethylated hardened castor oil<br>dextran (m.w. 40,000) | 100<br>2,000 | 94 | 92 |
| not added | - | 74 | 58 |

*Example 2*

To 10 μg of G-CSF, one of the stabilizing agents listed in Table 2 was added and the mixture was aseptically dissolved in a 20 mM phosphate buffer solution (containing 100 mM sodium chloride; pH, 7.4) to make a pharmaceutical preparation containing 10 μg of G-CSF per ml. The preparation was aseptically charged into a sulfa-treated glass vial and sealed to make a G-CSF solution. The time-dependent change in the activity of G-CSF in this solution was measured by the same method as used in Example 1 and the results are shown in Table 2.

Table 2

| Stabilizing agent | Amount (parts by weight) | Activity (%) | | |
|---|---|---|---|---|
| | | After storage at 4°C for 7 days | After storage at 4°C for 2 months | After storage at RT for 1 month |
| mannitol | 5,000 | 91 | 87 | 82 |
| hyaluronic acid | 2,000 | 93 | 87 | 70 |
| dextran (m.w. 40,000) | 2,000 | 96 | 95 | 85 |
| glycerin | 10,000 | 90 | 90 | 88 |
| neuraminic acid | 5,000 | 93 | 91 | 84 |
| chitin | 2,000 | 95 | 92 | 86 |
| dextrin | 2,000 | 90 | 92 | 87 |
| human serum albumin | 1,000 | 99 | 95 | 92 |
| human serum globulin | 1,000 | 98 | 94 | 90 |
| acid-treated gelatin | 2,000 | 97 | 96 | 87 |
| alkali-treated gelatin | 500 | 99 | 95 | 92 |
| collagen | 2,000 | 99 | 94 | 88 |
| polyethylene glycol (m.w. 4,000) | 10,000 | 94 | 89 | 90 |
| hydroxypropyl cellulose | 2,000 | 98 | 95 | 92 |
| sodium carboxymethyl cellulose | 2,000 | 92 | 91 | 80 |
| hydroxyethyl cellulose | 4,000 | 92 | 94 | 90 |
| polyvinyl alcohol (m.w. 50,000) | 4,000 | 97 | 93 | 90 |
| polyvinylpyrrolidone (m.w. 50,000) | 4,000 | 95 | 95 | 92 |
| sorbitan monolaurate | 400 | 97 | 96 | 95 |
| polyoxyethylene sorbitan monolaurate | 400 | 100 | 96 | 94 |

Table 2 (cont'd)

| Stabilizing agent | Amount (parts by weight) | Activity (%) | | |
|---|---|---|---|---|
| | | After storage at 4°C for 7 days | After storage at 4°C for 2 months | After storage at RT for 1 month |
| polyoxyethylene sorbitan monostearate | 400 | 98 | 97 | 94 |
| polyoxyethylene polyoxypropylene glycol ether | 400 | 100 | 94 | 93 |
| polyoxyethylated hardened castor oil | 400 | 99 | 98 | 90 |
| sodium lauryl sulfate | 2,000 | 97 | 93 | 87 |
| lecithin | 2,000 | 97 | 94 | 90 |
| human serum albumin<br>mannitol<br>cysteine | 2,000<br>2,000<br>100 | 100 | 99 | 97 |
| human serum albumin<br>polyoxyethylene sorbitan monolaurate<br>mannitol | 2,000<br>100<br>2,000 | 99 | 97 | 95 |
| human serum albumin<br>hydroxypropyl cellulose<br>dextran (m.w. 40,000) | 1,000<br>500<br>2,000 | 99 | 97 | 95 |
| polyoxyethylene sorbitan monopalmitate<br>sorbitol | 100<br>2,000 | 96 | 96 | 93 |
| polyoxyethylated hardened castor oil<br>dextran (m.w. 40,000) | 100<br>2,000 | 95 | 92 | 92 |
| not added | - | 72 | 61 | 47 |

*Example 3*

To 10 μg of G-CSF, one of the stabilizing agents listed in Table 3 was added and the mixture was aseptically dissolved in a 20 mM phosphate buffer solution (containing 100 mM sodium chloride; pH, 7.4) to make a pharmaceutical preparation containing 10 μg of G-CSF per ml. One milliliter of the preparation was charged into a sulfa-treated silicone-coated glass vial and left at 4°C. The effectiveness of each stabilizing agent in preventing G-CSF adsorption was evaluated by measuring the residual activity of G-CSF in the solution after 0.5, 2 and 24 hours. The measurement was conducted by method (b) using reverse-phase high-performance liquid chromatography. The results are shown in Table 3.

Table 3

| Stabilizing agent | Amount (parts by weight) | Residual activity (%) | | | |
|---|---|---|---|---|---|
| | | initial | 0.5 h | 2 h | 24 h |
| monnitol | 5,000 | 100 | 93 | 90 | 91 |
| hyaluronic acid | 2,000 | 100 | 97 | 92 | 92 |
| dextran (m.w. 40,000) | 2,000 | 100 | 98 | 95 | 96 |
| glycerin | 10,000 | 100 | 94 | 91 | 90 |
| heparin | 2,000 | 100 | 92 | 90 | 90 |
| glucuronic acid | 5,000 | 100 | 96 | 90 | 91 |
| ketoglycolic acid | 5,000 | 100 | 92 | 88 | 90 |
| human serum albumin | 1,000 | 100 | 100 | 101 | 99 |
| human serum globulin | 1,000 | 100 | 98 | 100 | 98 |
| alkali-treated gelatin | 500 | 100 | 99 | 98 | 99 |
| acid-treated gelatin | 2,000 | 100 | 99 | 97 | 97 |
| collagen | 2,000 | 100 | 100 | 98 | 99 |
| polyethylene glycol (m.w. 4,000) | 10,000 | 100 | 100 | 100 | 99 |
| hydroxypropyl cellulose | 2,000 | 100 | 100 | 100 | 99 |
| sodium carboxymethyl cellulose | 2,000 | 100 | 98 | 96 | 95 |
| hydroxyethyl cellulose | 4,000 | 100 | 96 | 93 | 92 |
| polyvinyl alcohol (m.w. 50,000) | 4,000 | 100 | 99 | 100 | 98 |
| polyvinylpyrrolidone (m.w. 50,000) | 4,000 | 100 | 98 | 98 | 96 |
| sorbitan monocaprylate | 400 | 100 | 100 | 100 | 98 |
| polyoxyethylene sorbitan monostearate | 400 | 100 | 100 | 98 | 100 |
| polyoxyethylated hardened castor oil | 400 | 100 | 99 | 101 | 99 |

Table 3 (cont'd)

| Stabilizing agent | Amount (parts by weight) | Residual activity (%) | | | |
|---|---|---|---|---|---|
| | | initial | 0.5 h | 2 h | 24 h |
| sodium lauryl sulfate | 2,000 | 100 | 100 | 99 | 97 |
| lecithin | 2,000 | 100 | 99 | 100 | 98 |
| human serum albumin<br>mannitol<br>cysteine | 2,000<br>2,000<br>100 | 100 | 100 | 100 | 101 |
| human serum albumin<br>polyoxyethylene sorbitan monolaurate<br>mannitol | 2,000<br>100<br>2,000 | 100 | 100 | 98 | 99 |
| human serum albumin<br>hydroxypropyl cellulose<br>dextran (m.w. 40,000) | 1,000<br>500<br>2,000 | 100 | 101 | 99 | 100 |
| polyoxyethylene sorbitan monolaurate<br>sorbitol | 100<br>2,000 | 100 | 100 | 99 | 99 |
| polyoxyethylated hardened castor oil<br>dextran (m.w. 40,000) | 100<br>2,000 | 100 | 100 | 98 | 97 |
| not added | - | 100 | 91 | 72 | 73 |

CLAIMS

1. A stable granulocyte colony stimulating factor containing pharmaceutical preparation that contains, in addition to the granulocyte colony stimulating factor present as the effective ingredient, at least one substance selected from the group consisting of a pharmaceutically acceptable surfactant, saccharide, protein and high-molecular weight compound.

2. A stable granulocyte colony stimulating factor containing pharmaceutical preparation according to Claim 1 which contains the surfactant in an amount of 1—10,000 parts by weight per part by weight of the granulocyte colony stimulating factor.

3. A stable granulocyte colony stimulating factor containing pharmaceutical preparation according to Claim 1 or 2 wherein said surfactant is at least one member selected from the group consisting of a nonionic surfactant, an anionic surfactant and a natural surfactant, the nonionic surfactant being a sorbitan aliphatic acid ester, a glycerin aliphatic acid ester, a polyglycerin aliphatic acid ester, a polyoxyethylene sorbitan aliphatic acid ester, a polyoxyethylene sorbitol aliphatic acid ester, a polyoxyethylene glycerin aliphatic acid ester, a polyethylene glycol aliphatic acid ester, a polyoxyethylene alkyl ether, a polyoxyethylene polyoxypropylene alkyl ether, a polyoxyethylene alkylphenyl ether, a polyoxyethylated hardened castor oil, a polyoxyethylated beeswax derivative, a polyoxyethylene lanolin derivative, or a polyoxyethylene aliphatic acid amide, the anionic surfactant being an alkyl sulfate salt, a polyoxyethylene alkyl ether sulfate salt, or an alkyl sulfosuccinate ester salt, and the natural surfactant being lecithin, glycerophospholipid, sphingophospholipid, or a sucrose aliphatic acid ester.

4. A stable granulocyte colony stimulating factor containing pharmaceutical preparation according to Claim 1 which contains the saccharide in an amount of 1—10,000 parts by weight per part by weight of the granulocyte colony stimulating factor.

5. A stable granulocyte colony stimulating factor containing pharmaceutical preparation according to Claim 1 or 4 wherein said saccharide is at least one member selected from the group consisting of glycerin, erythritol, arabitol, xylitol, sorbitol, mannitol, glucuronic acid, iduronic acid, galacturonic acid, neuraminic acid, glyconic acid, mannuronic acid, ketoglycolic acid, ketogalactonic acid, ketogulonic acid, hyaluronic acid and salts thereof, chondroitin sulfate and salts thereof, heparin, inulin, chitin and derivatives thereof, chitosan and derivatives thereof, dextrin, dextran with an average molecular weight of 5,000 -150,000, and alginic acid and salts thereof.

6. A stable granulocyte colony stimulating factor containing pharmaceutical preparation according to Claim 1 which contains the protein in an amount of 1—20,000 parts by weight per part by weight of the granulocyte colony stimulating factor.

7. A stable granulocyte colony stimulating factor containing pharmaceutical preparation according to Claim 1 or 6 wherein said protein is at least one member selected from the group consisting of human serum albumin, human serum globulin, gelatin, acid- or alkali-treated gelatin with an average molecular weight of 7,000—100,000, and collagen.

8. A stable granulocyte colony stimulating factor containing pharmaceutical preparation according to Claim 1 which contains the high-molecular weight compound in an amount of 1—20,000 parts by weight per part by weight of the granulocyte colony stimulating factor.

9. A stable granulocyte colony stimulating factor containing pharmaceutical preparation according to Claim 1 or 8 wherein said high-molecular weight compound is at least one member selected from the group consisting of hydroxypropyl cellulose, hydroxymethyl cellulose, sodium carboxymethyl cellulose, hydroxyethyl cellulose, polyethylene glycol with a molecular weight of 300—6,000, polyvinyl alcohol with a molecular weight of 20,000—100,000, and polyvinylpyrrolidone with a molecular weight of 20,000—100,000.

10. A process for producing a stable granulocyte colony stimulating factor containing pharmaceutical preparation that contains, in addition to the granulocyte colony stimulating factor present as the effective ingredient, at least one substance selected from the group consisting of a pharmaceutically acceptable surfactant, saccharide, protein and high-molecular weight compound.

11. A stable granulocyte colony stimulating factor containing pharmaceutical preparation substantially as hereinbefore described, with reference to Example 1, 2 or 3.

12. A process for producing a stable granulocyte colony stimulating factor containing pharmaceutical preparation substantially as hereinbefore described, with reference to Example 1, 2 or 3.

Published 1988 at The Patent Office, State House, 66/71 High Holborn, London WC1R 4TP. Further copies may be obtained from The Patent Office, Sales Branch, St Mary Cray, Orpington, Kent BR5 3RD. Printed by Burgess & Son (Abingdon) Ltd. Con. 1/87.